Fig. 1
TOP

Naive mRNA

Ab-Phage Library (a)

Single hybridoma gene

Pool of selected sequences (b)

Error-prone PCR (c)

Pool of selected sequences (e)

Select

Shuffling (d)

Pool of selected sequences (f)

Select

(g)

Template — PCR → DNAseI digest → Gel Purify 10-50 bp fragments → PCR without primers → PCR with primers → Clone into white pUC18 derivative →

LacZ alpha

pUC18++

a
1 kb

b
100 bp

c
50 bp
10 bp

d
25 30 35 40 45
cycles

e

84% blue colonies

FIG. 2

Figure 3

Template   Phenotype

LacZ alpha gene

pUC18 - +   White

pUC18 + -   White

BamHI Polylinker   XhoI NcoI   NheI EcrI   EcoO109

BamHI Polylinker   HindIII ScaI   HpaI EcrV   EcoO109

75 bp

HindIII NheI diagnostic fragment

SalI   XhoI NcoI   NheI   EcoRI   BssHII PvuI·

GTCGACCT·GACCATGGCCTAA·TAATTAACTAAT---·TACTCCAGGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCGCATCCACCTTTCGGCTAGCTGGCCGGAATTCCGAGAA---··CGGCG

SalI   PstI   SphI HindIII   PstI   BstEII   FspI

GTCGACCT·CAGGCCATGCAAGCTTAGCGACTTGCTGTAGT·TGACTGCAGGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCGCATCCACCTTTCGGCTAGT·TAA·TAATTAACTAA·TATATC·CGCGG

SalI   PstI   ScaI PstI   BstEII   FspI   HpaI   EcrV BssHII PvuI·

Figure 4

Figure 4 diagram showing LacZ alpha gene with restriction sites BamHI, XhoI, NcoI, NheI, EcrI, EcoO109, and the Spike region with HindIII and the HindIII-NheI diagnostic fragment.

Figure 5

A10B = scFv of anti-R-IgG antibody (Pharmacia)

mRNA → H chain, L chain → scFv → clone → express → select
                                        ↑
                          chain shuffle ←

scFv structure:

SfiI Leader FR1 CDR1 FR2 CDR2 FR3 CDR3 FR4 LINKER FR1 CDR1 FR2 CDR2 FR3 CDR3 FR4 NotI

←————— HEAVY CHAIN —————→   ←————— LIGHT CHAIN —————→

Total: 750 bp

First Experiment:

A10B scFv

SfiI   CDRH1   CDRH2   CDRH3   CDRL1   CDRL2   CDRL3   NotI

Spike: 70/10/10/10 CDRs:

                                    CDRL1
                    CDRL2   CDRL3
            CDRH1   CDRH2
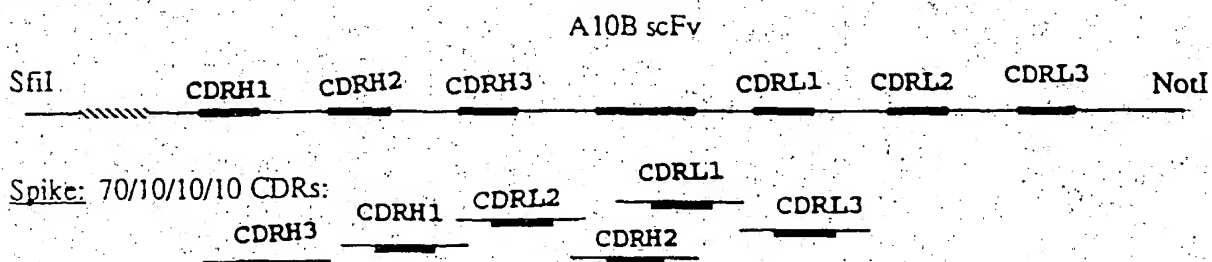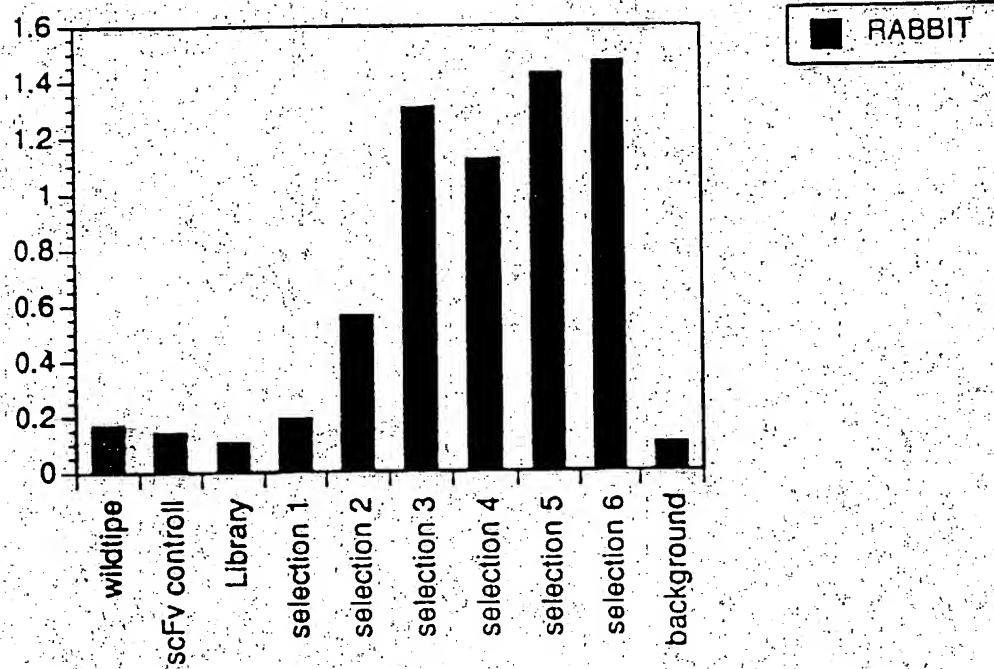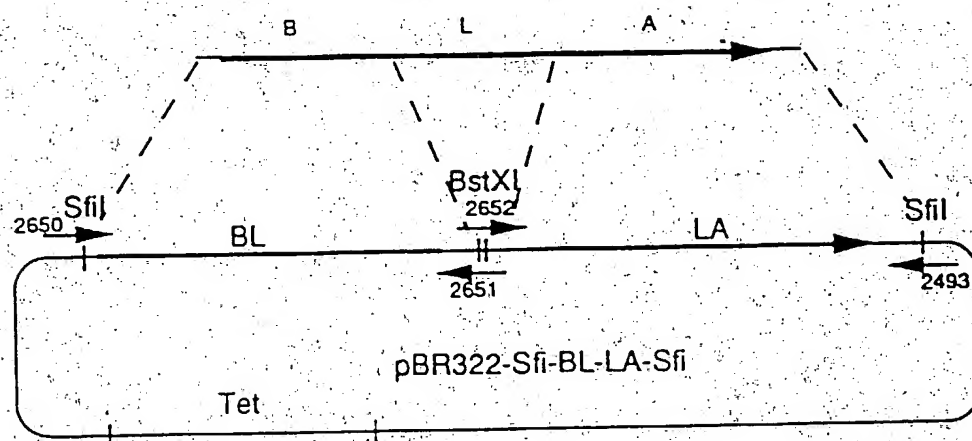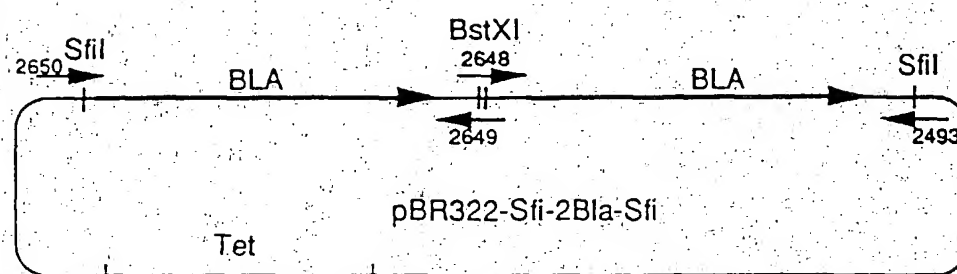   CDRH3
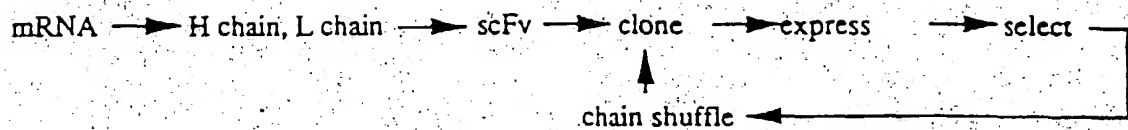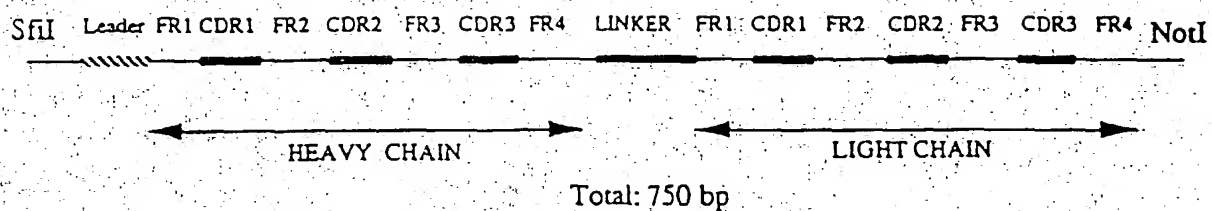
FIG. 6

FIG. 7

FIG. 8

In vivo recombination via direct repeats - Intraplasmidic

Cell	Tet colonies	Amp colonies	Colony PCR
TG-1	131	21	3/3 at 1 kb
JC8679	123	31	4/4 at 1 kb
vector control	51	0	

FIGURE 9

## In vivo recombination via direct repeats - Intraplasmidic

Cell	Tet colonies	Amp colonies	Colony PCR
TG-1	28	54	7/7 at 1 kb
JC8679	149	117	3/3 at 1 kb
vector control	51	0	

FIGURE 10

Homologous Recombination by Fragment Electroporation

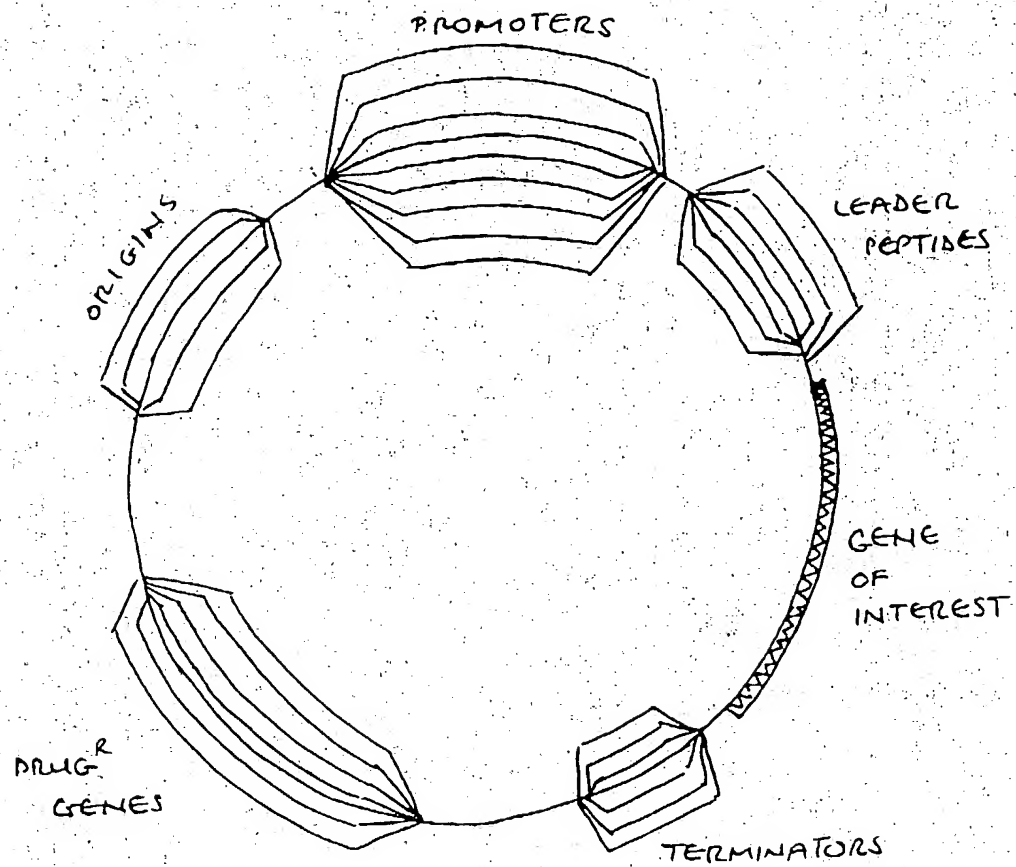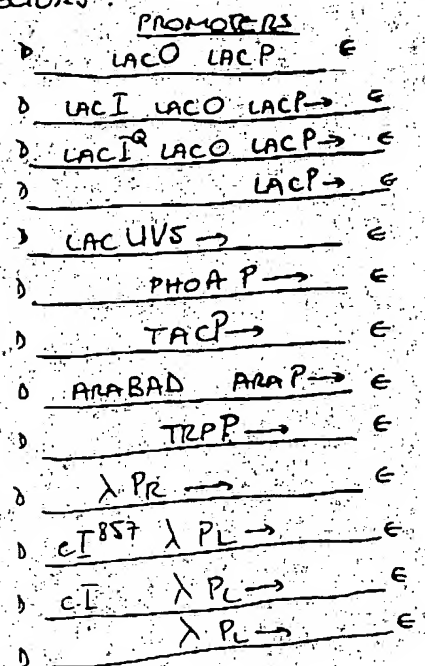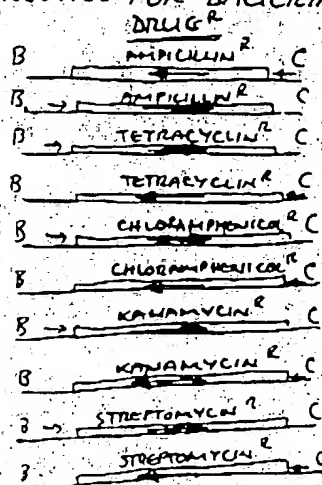| Approach	Amp colonies	Amp Tet colonies	% homologous recombination		Comment
1- cut vector 1 insert JC8679	4,000	1,500	100% (N=14)		Efficient insertion by homologous recombination with co-electroporated vector
2- cut vector 2 inserts JC8679	2,000	16	100% (N=2)		100x less efficient than 1 fragment
3- uncut vector 1 insert JC8679	16	0			Homologous insertion depends on free ends.
4- no vector 1 insert JO8679::pUCSfi-Sfi	5,000	10,000	70% (N=7)		If vector is in cells already, high efficiency occurs even though vector is uncut
5- no vector 1 insert JC8679	2,000	0			- control: non-homologous insertion into chromosome
6- cut vector no insert JC8679	N.D.	0			- control: No amp background
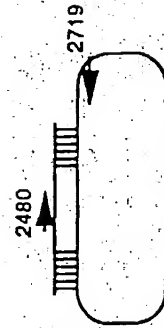
Homologous recombination colony PCR:

2480
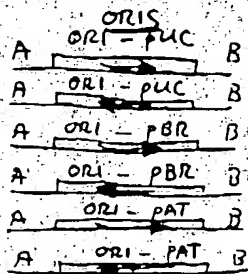
2719

FIGURE 11

FIGURE 12

EXAMPLES OF CASSETTES FOR BACTERIAL VECTORS:

ORIS

A	ORI – PUC	B
A	ORI – PUC	B
A	ORI – PBR	B
A	ORI – PBR	B
A	ORI – PAT	B
A	ORI – PAT	B

DRUG^R

B	AMPICILLIN^R	C
B	AMPICILLIN^R	C
B	TETRACYCLIN^R	C
B	TETRACYCLIN^R	C
B	CHLORAMPHENICOL^R	C
B	CHLORAMPHENICOL^R	C
B	KANAMYCIN^R	C
B	KANAMYCIN^R	C
B	STREPTOMYCIN^R	C
B	STREPTOMYCIN^R	C

PROMOTERS

D	LACO LACP	E
D	LAC I LACO LACP →	E
D	LAC I^Q LACO LACP →	E
D	LACP →	E
D	LAC UV5 →	E
D	PHOA P →	E
D	TAC P →	E
D	ARABAD ARA P →	E
D	TRP P →	E
D	λ P$_R$ →	E
D	cI^{857} λ P$_L$ →	E
D	cI λ P$_L$ →	E
D	λ P$_L$ →	E
D		

TERMINATORS

F	M13	G
F	ØX174	G
F	P22 OOP	G
F	TRP	G
F	S10	G

SS DNA ORI

M13

SIGNAL PEPTIDES

E	OMPF	F
E	PHOA	F
E	OMPA	F
E	SST II	F
E	PEL B	F
E	BLA	F

FIGURE 13